Data Evaluation Record on the bioaccumulation of fluopyram in fish

PMRA Submission Number {.....} EPA MRID Number 47372337

Data Requirement: PMRA Data Code:
EPA DP Barcode: D353319
OECD Data Point:
EPA Guideline: 850.1730

Test material:

Common name:	Fluopyram.
Chemical name:	
IUPAC name:	N-{2-[3-chloro-5-(trifluoromethyl)-2-pyridyl]ethyl}-α,α,α-trifluoro-o-toluamide. N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide.
CAS name:	N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)benzamide. Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl).
CAS No:	658066-35-4.
Synonyms:	AE C656948.
SMILES string:	C1(Cl)C(CCNC(=O)C2=C(C(F)(F)F)C=CC=C2)=NC=C(C(F)(F)F)C=1 (EPI Suite, v3.12 SMILES String from ISIS .MOL file).

Primary Reviewer: Kindra Bozicevich
Cambridge Environmental
Secondary Reviewer: Joan Harlin
Cambridge Environmental
QC Manager: Joan Gaidos
Cambridge Environmental
Final Reviewer: Mohammed Ruhman
EPA Reviewer

Signature: Kindra Bozicevich
Date: 12/03/08

Signature: Joan Harlin
Date: 12/03/08

Signature:
Date: 12/03/08

Signature:
Date: 12/10/08

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 080302

CITATION: Bruns, E. and E. Weber. 2008. [Pyridyl-2,6-^{14}C]-fluopyram- bioconcentration and biotransformation in fish (*Lepomis macrochirus*). Unpublished study performed, sponsored, and submitted by Bayer CropScience AG, Monheim, Germany. Bayer Crop Science AG report ID/Report No.: EBGMP116 (pp. 1, 9). Laboratory Project ID: E 244 3300-6. Experimental initiation August 24, 2007 and completion October 9, 2007 (p. 15). Final report issued March 6, 2008.

ABSTRACT

Laboratory Accumulation- Fish

The bioaccumulation of [pyridyl-2,6-^{14}C]-labeled N-{2-[3-chloro-5-(trifluoromethyl)-2-pyridyl]ethyl}-α,α,α-trifluoro-o-toluamide (fluopyram; AE C656948; radiochemical purity >99%), dissolved in dimethylformamide, was studied in bluegill sunfish (*Lepomis macrochirus*) under flow-through conditions. The study was conducted in two parts. Part 1 Bioconcentration and Depuration was a 42-day phase at nominal concentrations of 6.0 µg/L (low dose) and 60.0 µg/L (high dose), conducted to examine the bioconcentration and depuration of [^{14}C]fluopyram by bluegill sunfish. Part 2 Biotransformation was a 7- and 14-day exposure at a nominal concentration of 60.0 µg/L, conducted to investigate the biotransformation of [^{14}C]fluopyram in fish. This study was conducted in accordance with USEPA Subdivision E § 72-6 and Subdivision N § 165-4 guidelines, and in compliance with USEPA FIFRA GLP 40 CFR Part 160. The test system consisted of 100-L glass test aquaria. For Part 1, two test aquaria were treated with [^{14}C]fluopyram and one served as a control. Aerated, reconstituted dilution water was delivered into the test aquaria at an average rate of *ca.* 25 L/hour/aquarium, equivalent to a turnover rate of *ca*. 6 times over 24 hours. A total of 60 fish/test aquaria were exposed for 28 days at 23.5°C. Following exposure, a 14-day depuration phase was initiated by draining the aquarium water to a height of *ca*. 5 cm and then filling the aquarium with uncontaminated dilution water. The fish were then exposed for 14 days under flow-through conditions. For Part 2, one test aquarium was treated with [^{14}C]fluopyram. Fifteen fish were exposed for 7 days and 15 fish were exposed for 14 days at 23.5°C. A depuration phase was not conducted.

For Part 1, four fish were collected from each of the low-dose and high-dose test vessels after 1, 3, 7, 10, 14, 21, and 28 days of exposure and after 1, 3, 7, 10, and 14 days of depuration. The fish were separated into edible tissues and viscera/nonedible parts. Fish were sampled to measure total ^{14}C activity by LSC. Four additional fish were collected from each test aquarium after 0 and 28 days of exposure and after 14 days of depuration for determination of lipid content.

Triplicate water samples were collected from each test aquarium after -1, 0, 1, 3, 7, 10, 14, 21, and 28 days of exposure and after 1, 3, 7, 10, and 14 days of depuration, and were analyzed directly by LSC to determine total ^{14}C activity. Additional water samples were collected from the 60 µg/L treatment group after 0, 1, and 28 days of exposure for determination of transformation products. The samples were applied to a pre-conditioned C18 SPE cartridge, the residues were eluted with acetonitrile, and the eluate was concentrated prior to analysis using reversed phase HPLC. The identities of fluopyram and its transformation products were confirmed by co-chromatography with the following reference standards: fluopyram (AE C656948); AE C656948-ethyl-diol-GA (isomer 3) (BD3307P); AE C656948-pyridyl acetic acid (BD3307G); AE C656948-7-OH-phenol-GA (BD3307H); AE C656948-7-OH-GA (isomer 1+2) (BD3307I); AE C656948-7-OH-phenol-SA (BD3307J); AE C656948-phenol-GA (mixture with AE C656948-enol-GA) (BD3307N); AE C656948-enol-GA, 2 isomers (BD3307K); AE C656948-8-OH-GA (isomer 2); AE C656948-7-OH-phenol (BD33070); AE C656948-7-hydroxy

(BD3307L) (BD3307D); AE C656948-8-hydroxy (BD3307M1) (BD3307E); and AE C656948-picoline (BD3307Q1).

For Part 2, 15 fish were collected from the test aquarium after 7 and 14 days of exposure. The fish were separated into edible and viscera/nonedible parts, solvent-extracted, then applied to a C18 SPE cartridge. The eluates were combined, concentrated, and analyzed using HPLC analysis. The remaining samples were analyzed using LSC following combustion. For further identification of transformation products, viscera extracts (day 14) were fractionated by repeated HPLC. Nine fractions of transformation products were obtained using this method, from which six were subjected to LC-MS/MS analysis. Viscera extracts (day 14) were also subjected to enzymatic and acidic cleavage.

Triplicate water samples were collected from each test aquarium after -1, 0, 1, 3, 7, 10 and 14 days of exposure. Aliquots of each sample were analyzed directly by LSC. Additional water samples were collected after 7 and 14 days of exposure for determination of transformation products using HPLC analysis.

Water quality parameters were monitored and maintained throughout the study period. During the exposure and depuration phases, the water temperature ranged from 22.8-23.4°C in all treated test vessels and 22.8-23.2°C in the control samples. Dissolved oxygen concentrations ranged from 61-99% saturation and pH values ranged from 6.6-7.3. All measured TOC values did not exceed the concentration of organic carbon originating from fluopyram and from the solubilizing agent by >10 mg/L. No fish mortalities were observed in any of the test vessels.

During the 28-day exposure phase, the mean measured concentration of [pyridyl-2,6-^{14}C]fluopyram in the water was 5.98 ± 0.4 µg/L (range 5.39-6.62 µg/L) for the Part 1 low-dose study, 59.9 ± 3.6 µg/L (range 54.9-64.7 µg/L) for the Part 1 high-dose study, and 55.1 ± 9.0 µg/L (range 33.7-61.7 µg/L) for the Part 2 study. Radioactivity in the control tank water ranged from 19.2-78.6 µg/L.

For Part 1- Bioconcentration and Depuration. In the **low-dose** (6 µg/L) fish tissue samples, [pyridyl-2,6-^{14}C]fluopyram residues reached steady state after 28 days of exposure, with maximum mean edible tissue, nonedible tissue (viscera), and whole fish concentrations of 0.292 ± 0.101 mg/kg, 1.01 ± 0.12 mg/kg, and 0.581 ± 0.086 mg/kg, respectively. Reviewer-calculated maximum mean BCF values, based on total residues, were 49 for edible tissue, 169 for nonedible tissue, and 97 for whole fish, each on day 28. Using Origin™ non-linear kinetic modeling, the study authors estimated Kinetic Bioconcentration Factors (BCF_{TRR}) to be 47.6, 156.4, and 87.9 for edible tissue, nonedible tissue, and whole fish, respectively. The time to reach 95% of steady-state was estimated as 30.6, 8.1, and 14.8 days for edible tissue, nonedible tissue, and whole fish, respectively, and the $t_{(1/2)}$ for clearance was estimated as 7.1, 1.9, and 3.4 days, respectively. Uptake rate constants (K_U) of 4.67 ± 0.42, 58.2 ± 2.0, and 17.8 ± 1.1 were estimated for edible tissue, nonedible tissue, and whole fish, respectively; corresponding depuration rate constants (K_d) were estimated to be 0.098 ± 0.03, 0.37 ± 0.16, and 0.20 ± 0.08, respectively.

During the depuration phase, [pyridyl-2,6-^{14}C]fluopyram residues decreased from 0.154 ± 0.025, 0.39 ± 0.08, and 0.25 ± 0.047 mg/kg in edible tissue, nonedible tissue, and whole fish, respectively, at 1 day to 0.120 ± 0.028, 0.19 ± 0.03, and 0.15 ± 0.028 mg/kg at 14 days. At the end of the 14-day depuration phase, percent depuration was 58.9%, 81.2%, and 74.2% in edible tissue, nonedible tissue, and whole fish, respectively. Lipid content was not measured during depuration.

In the **high-dose** (60 µg/L) fish tissue samples, [pyridyl-2,6-^{14}C]fluopyram residues reached steady state after 28 days of exposure, with maximum mean edible tissue, nonedible tissue, and whole fish concentrations of 2.49 ± 0.20 mg/kg, 8.79 ± 1.00 mg/kg, and 4.75 ± 0.33 mg/kg, respectively. Reviewer-calculated maximum mean BCF values, based on total residues, were 42 for edible tissue, 147 for nonedible tissue, and 79 for whole fish, each on day 28. The study authors estimated Kinetic Bioconcentration Factors (BCF_{TRR}) to be 35.9, 121.6, and 65.7 for edible tissue, nonedible tissue, and whole fish, respectively. The time to reach 95% of steady-state was estimated as 18.1, 4.6, and 7.7 days for edible tissue, nonedible tissue, and whole fish, respectively, and the $t_{(1/2)}$ for clearance was estimated as 4.2, 1.1, and 1.8 days, respectively. Uptake rate constants (K_U) of 5.96 ± 0.57, 78.7 ± 3.62, and 25.6 ± 1.59 were estimated for edible tissue, nonedible tissue, and whole fish, respectively; corresponding depuration rate constants (K_d) were estimated to be 0.17 ± 0.06, 0.65 ± 0.26, and 0.39 ± 0.175, respectively.

During the depuration phase, [pyridyl-2,6-^{14}C]fluopyram residues decreased from 1.04 ± 0.17, 3.26 ± 0.64, and 1.90 ± 0.33 mg/kg in edible tissue, nonedible tissue, and whole fish, respectively, at 1 day to 0.71 ± 0.16, 1.31 ± 0.26, and 0.97 ± 0.19 mg/kg at 14 days. At the end of the 14-day depuration phase, percent depuration was 71.5%, 85.1%, and 79.6% in edible tissue, nonedible tissue, and whole fish, respectively. Lipid content was not measured during depuration.

Based on HPLC analysis, [pyridyl-2,6-^{14}C]fluopyram accounted for >97% of the TRR in all water samples. In samples collected during the later exposure phase of fish, transformation product AE C656948-7-hydroxy was detected at *ca*. 1% of the TRR.

Mean lipid content was an average of 0.75 g (6.50% of the fish body weight) at Day 0, 0.91 g (7.56%) at Day 28, and 0.98 g (9.92%) on Day 42. The study authors calculated steady-state BCF value for fluopyram (whole fish, normalized to 6% lipid content) was 16.

For Part 2-Biotransformation. HPLC analysis of day 7 and day 14 edible tissues and viscera showed that 95.1-97.5% of the TRR was extractable. Fluopyram accounted for 24.7-54.6% and 11.0-21.9% of TRR in the organic extracts from edible and nonedible tissues, respectively. The study authors calculated steady-state BCF value for fluopyram (based on whole fish, wet weight) was 18. Six transformation products were isolated and identified. AE C656948-7-hydroxy accounted for maximums of 17.8% (day 7) and 6.8% (day 14) of the TRR from edible tissues and viscera, respectively. AE C656948-7-OH-GA (isomer 1) accounted for maximums of 3.9% (day 14) and 17.6% (day 7) of the TRR from edible tissues and viscera, respectively. AE

C656948-8-hydroxy accounted for maximums of 1.9% (day 7) and 1.8% (day 7) of the TRR from edible tissues and viscera, respectively. AE C656948-8-OH-GA (isomer 2), AE C656948-7-OH-GA (isomer 2), and AE C656948-pyridyl acetic acid were maximums of 3.1% (day 7), 7.2% (day 14), and 0.9% (day 7) of the TRR in the viscera, and were not detected in the edible tissues.

In addition, three unidentified compounds "characterized by LC-MS/MS" were isolated. BD3319D was a maximum of 5.1% of the TRR (day 14) in the viscera and was not detected in edible tissues. BD3319D3 was a maximum of 2.9% (day 14) and 5.8% (day 14) of the TRR from edible tissues and viscera, respectively. BD3319D8 was a maximum of 1.6% (day 14) and 5.0% (day 7) of the TRR from edible tissues and viscera, respectively.

The remaining residues (*ca*. 21-45% of the TRR) were characterized by their behavior during extraction, clean-up, and HPLC analysis and by enzymatic cleavage and by acid hydrolysis. Residues eluting in the column dead time of the HPLC profile accounted for *ca*. 6-11% of the TRR. The transformation products characterized accounted for 3-15 peaks in the HPLC profiles, with no single peak accounting for >7.4% of the TRR.

Based on HPLC analysis, [pyridyl-2,6-^{14}C]fluopyram accounted for >97% of the TRR in all water samples. In samples collected during the later exposure phase of fish, transformation product AE C656948-7-hydroxy was detected at *ca*. 1-2% of the TRR.

Study Acceptability: This study is classified as acceptable. No significant deviations from good scientific practices were noted.

MATERIALS AND METHODS

The study was conducted in two parts (p. 14). Part 1 was a 42-day phase to examine the bioconcentration and depuration of [pyridyl-2,6-^{14}C]fluopyram (N-{2-[3-chloro-5-(trifluoromethyl)-2-pyridyl]ethyl}-α,α,α-trifluoro-o-toluamide) by bluegill sunfish. Part 2 was a 7-14 day exposure to investigate the biotransformation of [pyridyl-2,6-^{14}C]fluopyram in fish.

For Part 1- Bioconcentration and Depuration. Bluegill sunfish (*Lepomis macrochirus*; Osage Catfisheries, Inc., Osage Beach, Missouri; lot F 2/07 A) were fed standard fish-feed daily (Pro Aqua Brutfutter, Skretting, Germany), based on 1.5-2% of average body weight, and acclimated in culture tanks on a 16-hour daylight photoperiod for a minimum of 14 days prior to test initiation (p. 17). The fish were treated with oxytetracyclin-hydrochloride (4 g/100 L) immediately following arrival at the test facility. At the onset of the study, the fish had a mean body weight of 8.6 ± 2.2 g and a mean body length of 7.4 ± 0.6 cm (p. 19). The organism loading at study initiation was 5.2 g fish/L and 0.86 g fish/L/day.

Continuous flow-through aquatic exposure system were prepared using three 100-L glass test aquaria (one control, two treated) maintained at 23.5°C (p. 18). [Pyridyl-2,6-^{14}C]fluopyram

(radiochemical and chemical purities >99%, Sample ID: KATH 6022; water solubility 16 mg/L at pH 7; p. 16), dissolved in dimethylformamide at nominal test concentrations of 6 µg/L and 60 µg/L, and dilution water, was delivered to 2000-mL mixing cells using a dosing system comprised of a ProMinentR mikro g/5a dispenser and flow-meters. The mixture was continuously running into the test aquaria at a rate of 2.5 mL/hour. Aerated, reconstituted dilution water was transferred into the test aquaria at an average rate of *ca.* 25 L/hour/aquarium. This amount was sufficient to replace the *ca*. 100 L test volume *ca*. 6 times within a 24-hour period.

For the exposure phase, a total of 60 randomly selected fish were transferred to each of the test and control aquaria and exposed for 28 days (p. 19). The fish were fed daily based on 1.5-2% of average body weight (p. 17). Measurements of dissolved oxygen concentration, temperature, pH, and total organic carbon (TOC) were measured initially and once a week thereafter (p. 21).

During the exposure period, four fish were collected from each test aquarium at 1, 3, 7, 10, 14, 21, and 28 days (p. 19; Table 1, p. 33). The fish were separated into edible (body, muscle, and skin, skeleton) and viscera/nonedible (head, fins, and internal organs) parts. Four additional fish were collected from each test aquarium at 0 and 28 days for determination of lipid content (p. 20). Triplicate water samples (10 mL) were collected from each test aquarium on each sampling day. The test aquaria were cleaned daily during working days (p. 21).

For the 14-day depuration period, the test aquaria were drained to a water height of *ca*. 5 cm and then filled with uncontaminated dilution water (p. 19). Four fish were collected from each test aquarium at 1, 3, 7, 10, and 14 days and processed as previously described (Table 1, p. 33). Four additional fish were collected from each test aquarium after 14 days of depuration for determination of lipid content (p. 20). Triplicate water samples (10 mL) were collected from each test aquarium on each sampling day. An additional water sample (500 mL) was collected from the 60 µg/L treatment group for determination of transformation products. The sample was stored deep-frozen prior to analysis.

For Part 2- Biotransformation. Bluegill sunfish (lot F 2/07) were fed standard fish-feed daily based on 1% of average body weight and acclimated in culture tanks as previously described (p. 17). At the onset of the study, the fish had a mean body weight of 22.4 ± 3.9 g and a mean body length of 10.1 ± 0.6 cm (p. 19). The organism loading at study initiation was 1.1 g fish/L/day.

A continuous flow-through aquatic exposure system was prepared using one 100-L glass test aquarium maintained at 23.5°C (p. 18). [Pyridyl-2,6-^{14}C]fluopyram (radiochemical purity >99%, Sample ID: KATH 6022; p. 16), dissolved in dimethylformamide at a nominal test concentration of 60 µg/L, and dilution water were delivered to the test aquarium as previously described.

For the exposure phase, 15 fish were exposed for 7 days and 15 fish were exposed for 14 days (p. 19). The fish were fed daily based on 1% of average body weight (p. 17). Measurements of dissolved oxygen concentration, temperature, pH, and total organic carbon (TOC) were measured initially and once a week thereafter (p. 21).

During the exposure period, 15 fish were collected from the test aquarium at 7 and 14 days and processed as previously described (p. 20; Table 1, p. 33). Triplicate water samples (10 mL) were collected on each sampling day. The test aquarium was cleaned daily during working days (p. 21). An additional water sample (500 mL) was collected for determination of transformation products. The water sample was stored deep-frozen prior to analysis.

Water: For Part 1- Bioconcentration and Depuration, triplicate water samples (10 mL) were collected after -1, 0, 1, 3, 7, 10, 14, 21, and 28 days of exposure and after 1, 3, 7, 10, and 14 days of depuration (p. 20, Table 1, p. 34). For Part 2- Biotransformation, triplicate water samples (10 mL) were collected after -1, 0, 1, 3, 7, 10 and 14 days of exposure. Aliquots of each sample were analyzed directly by LSC to determine the total radioactivity (p. 21). Method detection limits were not reported.

For identification of residues, additional water samples were collected from the 60 µg/L treatment group after 0, 1, and 28 days of exposure for Part 1- Bioconcentration and Depuration (p. 21; Appendix F, pp. 88-89; Appendices 2-6, pp. 150-154). For Part 2- Biotransformation, water samples were collected after 7 and 14 days of exposure. The samples (*ca*. 1000 mL) were applied to a pre-conditioned C18 SPE cartridge to concentrate the radioactive compounds. The retained radioactive residues were eluted with acetonitrile (250-300 mL), and the eluate was concentrated with a rotary evaporator prior to analysis using reversed phase HPLC under the following conditions: Purospher Star RP18 endcapped column (250 x 4.6 mm, 5 µm) with pre-column (4 x 4 mm), gradient mobile phase consisting of (A) water:25% aqueous ammonia:formic acid (adjusted to pH 7) and (B) acetonitrile:methanol (1:1, v:v) [percent A:B (v:v); 0-5 min., 100:0; 7-30 min., 90:10; 32-45 min., 85:15; 50 min., 77:23; 52 min., 68:32; 60-75 min., 57:43; 85 min., 30:70; 105-115 min., 0:100; 120-140 min., 100:0], flow rate 1.0 mL/min., with UV (270 nm) and radio detection (Appendix F, pp. 87-88). Column recovery was 98.5%.

The identities of fluopyram and its transformation products were confirmed by co-chromatography with the following reference standards (Appendix F, pp. 85, 90-91; Table 1, pp. 98-100; Figure 1, pp. 106-107):

Report name	**Other names**	**Peak No.** **Retention time (minutes)**
AE C656948	Fluopyram	Peak 25 91-92
AE C656948-Ethyl-diol-GA (isomer 3) (BD3307P)	BN178009	40
AE C656948-Pyridyl acetic acid (BD3307G)	BN178010	Peak 3 45-47
AE C656948-7-OH-phenol-GA (BD3307H)	BN178015	64
AE C656948-7-OH-GA (isomer 1+2) (BD3307I)	BN178017 (= isomer 1)	Peak 12 Peak 13 66-67 67-68
AE C656948-7-OH-phenol-SA (BD3307J)	BN178022	71
AE C656948-phenol-GA (mixture with AE C656948-enol-GA) (BD3307N)	BN153007	70 71 77
AE C656948-enol-GA, 2 isomers (BD3307K)	BN178025	71 78
AE C656948-8-OH-GA (isomer 2)	None reproted.	Peak 18 76-78
AE C656948-7-OH-phenol (BD33070)	BN178026	82
AE C656948-7-hydroxy (BD3307L) (BD3307D)	BN16604A	Peak 23 87
AE C656948-8-hydroxy (BD3307M1) (BD3307E)	BN16608A	Peak 24 88.5
AE C656948-picoline (BD3307Q1)	KML3627	89

Fish: For Part 1- Bioconcentration and Depuration, fish (4) were collected from each test aquarium at 1, 3, 7, 10, 14, 21, and 28 days of exposure and at 1, 3, 7, 10, and 14 days of depuration (p. 19; Table 1, p. 33). For Part 2- Biotransformation, 15 fish were collected from the test aquarium at 7 and 14 days of exposure (p. 20). The samples were transferred into pre-weighed vials, weighed, and treated with 20 mL of BTS-450 (Beckman Tissue-Solubilizer-450). The samples were incubated in a drying oven at 50°C and slightly agitated once daily. After a minimum of 5 days of incubation, duplicate aliquots were removed, mixed with 5N HCl (200 µL), and shaken prior to analysis using LSC. Method detection limits were not reported.

For Part 1- Bioconcentration and Depuration, four additional fish were collected from each test aquarium at 0 and 28 days of exposure and 14 days of depuration for determination of lipid content (p. 20; Appendix C, pp. 69-71). For this determination, whole fish (5 g) were homogenized with chloroform (5 mL) and methanol (10 mL) at a ratio of 1:1:2 (w:v:v). The suspension was diluted with chloroform (5 mL) and water (5 mL), and homogenized again. Following centrifugation, the supernatants were decanted. The organic phase was evaporated to a constant weight and the weight of the remainder was recorded as total lipids of the starting sample.

For Part 2- Biotransformation, 15 fish were collected from the test aquarium after 7 and 14 days of exposure (p. 20; Appendix F, p. 89). The fish were separated into edible (body, muscle, and skin, skeleton) and viscera/nonedible (head, fins, and internal organs) parts. The coarse pieces were combined, mixed thoroughly, and subsamples (*ca*. 60 g edibles; *ca*. 40-50 g viscera) were extracted three times with acetonitrile:water (80:20, v:v) using an Ultra Turrax homogenizer (p. 90). The suspensions were centrifuged and the supernatants were combined and applied to a C18 SPE cartridge washed with acetonitrile:water (8:2, v:v) and methanol:dichloromethane (1:1, v:v). The eluates were combined, concentrated to *ca*. 20-50 mL, and analyzed using HPLC as previously described. The remaining samples were dried at room temperature for *ca*. 3-4 days and analyzed using LSC following combustion.

For identification of residues, fish extracts obtained from Part 2- Biotransformation were analyzed using HPLC as previously described (Appendix F, p. 90). For further identification of transformation products, fish viscera extracts (day 14) were fractionated by repeated HPLC to isolate the major constituents of the sample (Appendix F, p. 91). Nine fractions of transformation products were obtained using the method, from which six were subjected to LC-MS/MS (Appendix F, p. 133, Figure 24, p. 133). LC-MS/MS was conducted using the following methods (Appendix F, p. 88):

HPLC Instrument and Setup	Solvents	Gradient
MS Instrument: TSQ 7000		
HPLC: Agilent HP 1100. Column: LiChrospher 60 RP Select B (250 x 2 mm; 5 µm). Flow: 0.2 mL/min.	A: 0.1% Formic acid in water. B: 0.1% Formic acid in acetonitrile.	0-1 min, 5%B. 25-35 min., 95%B.
MS Instrument: TSQ Quantum Ultra AM		
HPLC: Agilent HP 1100. Column: Nucleodur C18 Gravity (250 x 2 mm; 3 µm). Flow: 0.2 mL/min.	A: 0.1% Formic acid in water. B: 0.1% Formic acid in acetonitrile.	0-1 min, 5%B. 25-35 min., 95%B.

For enzymatic cleavage of conjugates, the fish viscera extract (day 14) obtained from Part 2- Biotransformation was incubated with pH 6 buffer solution and a mixture of β-glucuronidase/arylsulfatase from helix pomatia for 4 hours at 37°C (Appendix F, p. 91). The mixture was analyzed using HPLC analysis as previously described following incubation.

For acidic cleavage of conjugates, the fish viscera extract (day 14) obtained from Part 2- Biotransformation was mixed with 5N hydrochloric acid in a ratio that resulted in a 2N concentration of acid (Appendix F, p. 91). The mixture was heated for *ca*. 1-2 hours at 100°C and analyzed using HPLC as previously described. Identification of cleavage products was performed by chromatographic comparison and co-chromatography of the hydrolyzed samples with authentic reference compounds.

RESULTS AND DISCUSSION

Water quality parameters were monitored and maintained throughout the study period (pp. 25-26; Table 22, p. 56). During the exposure and depuration phases, the water temperature ranged from 22.8-23.4°C in all treated test vessels and 22.8-23.2°C in the control samples. Dissolved oxygen concentrations ranged from 61-99% saturation and pH values ranged from 6.6-7.3. All measured TOC values did not exceed the concentration of organic carbon originating from fluopyram and from the solubilizing agent by >10 mg/L (Table 23, p. 57). No fish mortalities were observed in any of the test vessels (p. 26; Table 21, pp. 54-55).

During the 28-day exposure phase, the mean measured concentration of [pyridyl-2,6-^{14}C]fluopyram in the water was 5.98 ± 0.4 µg/L (range 5.39-6.62 µg/L) for the Part-1 low-dose study, 59.9 ± 3.6 µg/L (range 54.9-64.7 µg/L) for the Part-1 high-dose study, and 55.1 ± 9.0 µg/L (range 33.7-61.7 µg/L) for the Part-2 study (p. 26; Table 5, pp. 35-36). Radioactivity in the control tank water ranged from 19.2-78.6 µg/L.

For Part 1- Bioconcentration and Depuration. In the **low-dose** (6 µg/L) fish tissue samples, [pyridyl-2,6-^{14}C]fluopyram residues reached steady state after 28 days of exposure, with maximum mean edible tissue, nonedible tissue, and whole fish concentrations of 0.292 ± 0.101 mg/kg, 1.01 ± 0.12 mg/kg, and 0.581 ± 0.086 mg/kg, respectively (p. 28; Tables 9-11, pp. 43-45). Reviewer-calculated maximum mean BCF values, based on total residues, were 49 for edible tissue, 169 for nonedible tissue, and 97 for whole fish, each at day 28 (DER Attachment 2; Reviewer's Comment). The study authors calculated maximum mean BCF values of 48.8 ± 16.9 (day 28), 171.1 ± 7.9 (day 10), and 97.2 ± 14.4 (day 28) for edible tissue, nonedible tissue, and whole fish, respectively, based on total radioactivity (p. 29; Tables 12-14, pp. 46-48). Using Origin™ non-linear kinetic modeling, the study authors estimated Kinetic Bioconcentration Factors (BCF_{TRR}) to be 47.6, 156.4, and 87.9, for edible tissue, nonedible tissue, and whole fish, respectively. The time to reach 95% of steady-state was estimated as 30.6, 8.1, and 14.8 days for edible tissue, nonedible tissue, and whole fish, respectively; the $t_{(1/2)}$ for clearance was estimated as 7.1, 1.9, and 3.4 days, respectively (Figures 1-3, pp. 58-60). Uptake rate constants (K_U) of 4.67 ± 0.42, 58.2 ± 2.0, and 17.8 ± 1.1 were estimated for edible tissue, nonedible tissue, and whole fish, respectively; corresponding depuration rate constants (K_d) were estimated to be 0.098 ± 0.03, 0.37 ± 0.16, and 0.20 ± 0.08, respectively.

During the depuration phase, [pyridyl-2,6-^{14}C]fluopyram residues decreased from 0.154 ± 0.025, 0.39 ± 0.08, and 0.25 ± 0.047 mg/kg in edible, nonedible and whole fish, respectively, at 1 day to 0.120 ± 0.028, 0.19 ± 0.03, and 0.15 ± 0.028 mg/kg at 14 days (Tables 9-11, pp. 43-45). At the end of the 14-day depuration phase, percent depuration was 58.9%, 81.2%, and 74.2% in edible tissue, nonedible tissue, and whole fish, respectively (DER Attachment 2). Lipid content was not measured during depuration.

In the **high-dose** (60 µg/L) fish tissue samples, [pyridyl-2,6-^{14}C]fluopyram residues reached steady state after 28 days of exposure, with maximum mean edible tissue, nonedible tissue, and

whole fish concentrations of 2.49 ± 0.20 mg/kg, 8.79 ± 1.00 mg/kg, and 4.75 ± 0.33 mg/kg, respectively (p. 28; Tables 9-11, pp. 43-45). Reviewer-calculated maximum mean BCF values, based on total residues, were 42 for edible tissue, 147 for nonedible tissue, and 79 for whole fish, each on day 28 (DER Attachment 2; Reviewers Comment). The study authors calculated maximum mean BCF values of 41.6 ± 3.3, 146.6 ± 16.7, and 79.2 ± 5.5 for edible tissue, nonedible tissue, and whole fish, each on day 28, based on total radioactivity (p. 29; Tables 12-14, pp. 46-48). Using Origin™ non-linear kinetic modeling, the study authors estimated Kinetic Bioconcentration Factors (BCF_{TRR}) to be 35.9, 121.6, and 65.7 for edible tissue, nonedible tissue, and whole fish, respectively. The time to reach 95% of steady-state was estimated as 18.1, 4.6, and 7.7 days for edible tissue, nonedible tissue, and whole fish, respectively, and the $t_{(1/2)}$ for clearance was estimated as 4.2, 1.1, and 1.8 days, respectively(Figures 4-6, pp. 61-63). Uptake rate constants (K_U) of 5.96 ± 0.57, 78.7 ± 3.62, and 25.6 ± 1.59, were estimated for edible tissue, nonedible tissue, and whole fish, respectively; corresponding depuration rate constants (K_d) were estimated to be and 0.17 ± 0.06, 0.65 ± 0.26, 0.39 ± 0.175, respectively.

During the depuration phase, [pyridyl-2,6-^{14}C]fluopyram residues decreased from 1.04 ± 0.17, 3.26 ± 0.64, and 1.90 ± 0.33 mg/kg in edible tissue, nonedible tissue, and whole fish, respectively, at 1 day to 0.71 ± 0.16, 1.31 ± 0.26, and 0.97 ± 0.19 mg/kg at 14 days (Tables 9-11, pp. 43-45). At the end of the 14-day depuration phase, percent depuration was 71.5%, 85.1%, and 79.6% in edible tissue, nonedible tissue, and whole fish, respectively (DER Attachment 2). Lipid content was not measured during depuration.

For Part 2-Biotransformation. HPLC analysis of day 7 and day 14 edible tissues and viscera showed that 95.1-97.5% of the TRR was extractable (p. 27; Appendix F. p. 92; Table 5, p. 103; Figures 16-19, pp. 122-128; Appendices 7-10, pp. 155-162). Fluopyram accounted for 24.7-54.6% and 11.0-21.9% of TRR in the organic extracts from edible tissues and viscera, respectively. The study authors calculated steady-state BCF value for fluopyram (based on whole fish, wet weight) was 18 (p. 29). Six transformation products were isolated and identified. AE C656948-7-hydroxy accounted for maximums of 17.8% (day 7) and 6.8% (day 14) of the TRR from edible tissues and viscera, respectively. AE C656948-7-OH-GA (isomer 1) accounted for maximums of 3.9% (day 14) and 17.6% (day 7) of the TRR from edible tissues and viscera, respectively. AE C656948-8-hydroxy accounted for maximums of 1.9% (day 7) and 1.8% (day 7) of the TRR from edible tissues and viscera, respectively. AE C656948-8-OH-GA (isomer 2), AE C656948-7-OH-GA (isomer 2), and AE C656948-pyridyl acetic acid were maximums of 3.1% (day 7), 7.2% (day 14), and 0.9% (day 7) of the TRR in the viscera and were not detected in the edible tissues.

Further identification of [^{14}C]compounds in day 14 viscera extracts was performed by comparison of the metabolic profile with the profile of rat urine and rat bile, and by comparison with reference standards (Appendix F, p. 93; Figures 21-22, pp. 130-131). The identities of fluopyram and its transformation products were confirmed using HPLC and LC-MS/MS analyses (Appendix F, Figures 23-24, pp. 132-133; Figures 26-27, pp. 136-138; Figure 30, pp. 142-143). In addition to the transformation products addressed previously, three unidentified compounds “characterized by LC-MS/MS” were isolated. BD3319D was a maximum of 5.1%

of the TRR (day 14) in the viscera, and was not detected in edible tissues (p. 27; Appendix F, Table 5, p. 103; Figure 25, pp. 134-135). BD3319D3 was a maximum of 2.9% (day 14) and 5.8% (day 14) of the TRR from edible tissues and viscera, respectively (Figure 28, pp. 139-140). BD3319D8 was a maximum of 1.6% (day 14) and 5.0% (day 7) of the TRR from edible tissues and viscera, respectively (Figure 29, p. 141).

The remaining residues (*ca*. 21-45% of the TRR) were characterized by their behavior during extraction, clean-up, and HPLC analysis and by enzymatic cleavage and by acid hydrolysis (p. 27; Appendix F, pp. 93-94; Appendix F, Figures 31-32, pp. 144-145). Residues eluting in the column dead time of the HPLC profile accounted for *ca*. 6-11% of the TRR and were probably represented by matrix associated transformation products. The transformation products characterized accounted for 3-15 peaks in the HPLC profiles, with no single peak accounting for >7.4% of the TRR (except the peak in the column dead time for the samples of edibles).

Lipid analysis: For Part 1- Bioconcentration and Depuration, lipid analysis was conducted on fish collected after 0 and 28 days of exposure and after 14 days of depuration (p. 20). Mean lipid content was an average of 0.75 g (6.50% of the fish body weight) at Day 0, 0.91 g (7.56%) at Day 28, and 0.98 g (9.92%) on Day 42 (Tables 19-20, pp. 52-53; Appendix C, Table C1, p. 71). The study authors calculated steady-state BCF value for fluopyram (whole fish, normalized to 6% lipid content) was 16 (p. 29).

Water extract analysis: Based on HPLC analysis for Part-1 Bioconcentration and Depuration and Part 2 Biotransformation, [pyridyl-2,6-^{14}C]fluopyram accounted for >97% of the TRR in all extracted water samples (p. 27; Appendix F, p. 92; Table 4, p. 102; Figures 9-13, pp. 115-119). In samples collected during the later exposure phase of fish, transformation product AE C656948-7-hydroxy was detected at *ca*. 1-2% of the TRR (Appendix F, Figure 15, p. 121).

DEFICIENCIES/DEVIATIONS

No significant deviations from good scientific practices were noted.

REVIEWER'S COMMENTS

1. The study was conducted according to USEPA Subdivision E § 72-6 (1982); USEPA Subdivision N § 165-4 (1982); USEPA "Toxic Substances Control, Discussion of Pre-manufacture Testing Policy and Technical Issues, Request for Comment", Federal Register, Vol. 44, No. 53, 16284-16292 (1979); OECD Guideline No. 305 (1996); and ASTM Standard E 1022-84 (1988; pp. 9, 14, 18, 31). The study was conducted in compliance with USEPA FIFRA GLP 40 CFR Part 160; OECD Principles of GLP; Annex 1 of the German chemical law (ChemG; 2002); and Japanese Ministry of Agriculture, Forestry, and Fisheries (1999; p. 3; Appendix D, pp. 72-73; Appendix F, pp. 163-164). Signed and dated Data

Confidentiality, GLP, Quality Assurance, and Certificate of Authenticity statements were provided (pp. 2-5).

2. The reviewer calculated the maximum bioconcentration factors for total radioactive residues (TRR) in edible tissues, viscera, and whole fish using the following equation:

   maximum concentration of TRR in tissue (µg/kg) ÷
   average concentrations of total residues in the water through the relevant interval (µg/L).

3. Method detection limits for LSC and HPLC analyses of water and fish samples were not reported.

4. The study authors provided a proposed transformation pathway for fluopyram in fish (p. 12; Appendix F, p. 82; Figure 34, p. 147).

5. HPLC analysis was used to demonstrate the stability of [pyridyl-2,6-^{14}C]fluopyram in the stock solutions used for all tests (Appendix F, p. 92; Table 3, p. 102; Figures 2-7, pp. 108-113). [Pyridyl-2,6-^{14}C]fluopyram was demonstrated to be sufficiently stable throughout the study period.

6. Prior to analysis, the fish were stored frozen at *ca*. -18C immediately following sacrifice (Appendix F, p. 94). All analyses were completed within less than 4 weeks after the sacrifice.

Attachment 1: Structures of Parent Compound and Transformation Products

Fluopyram [AE C656948]

IUPAC Name: N-{2-[3-chloro-5-(trifluoromethyl)-2-pyridyl]ethyl}-α,α,α-trifluoro-o-toluamide.
N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide.

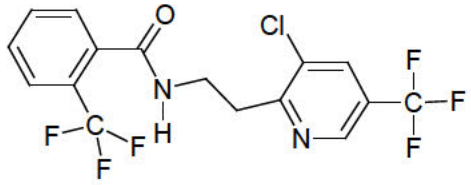
CAS Name: N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)benzamide.
Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl).

CAS Number: 658066-35-4.

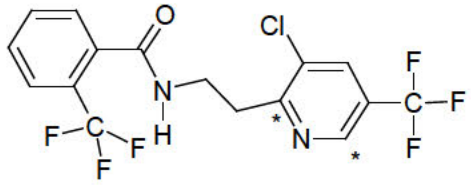
SMILES String: C1(Cl)C(CCNC(=O)C2=C(C(F)(F)F)C=CC=C2)=NC=C(C(F)(F)F)C=1 (EPI Suite, v3.12 SMILES String from ISIS .MOL file).

Empirical formula: $C_{16}H_{11}ClF_6N_2O$ **Molecular formula:** $C_{16}H_{11}ClF_6N_2O$

Unlabeled



[Pyridyl-2,6-^{14}C]Fluopyram



* = Location of the radiolabel.

Identified Compounds
(Parent and Transformation Products)

Parent

Fluopyram [AE C656948]

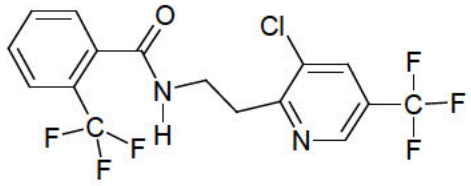
IUPAC Name: N-{2-[3-chloro-5-(trifluoromethyl)-2-pyridyl]ethyl}-α,α,α-trifluoro-o-toluamide.
N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide.

CAS Name: N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)benzamide.
Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)-(9Cl).

CAS Number: 658066-35-4.

SMILES String: C1(Cl)C(CCNC(=O)C2=C(C(F)(F)F)C=CC=C2)=NC=C(C(F)(F)F)C=1 (EPI Suite, v3.12 SMILES String from ISIS .MOL file).

Empirical formula: $C_{16}H_{11}ClF_6N_2O$ **Molecular formula:** $C_{16}H_{11}ClF_6N_2O$



Transformation Products

AE C656948-7-hydroxy [BD3307L, BD3307D, BN16604A, HF3605F5]

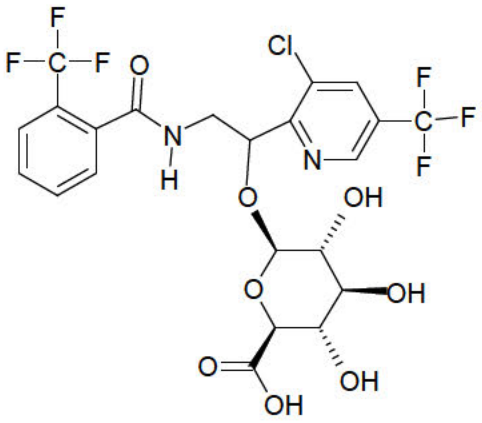
IUPAC Name: N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]-2-hydroxyethyl}-2-(trifluoromethyl)benzamide (ISIS/Draw with ACD/Name add-in).

CAS Name: Not reported.

CAS Number: Not reported.

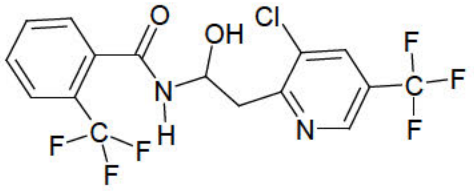
AE C656948-7-OH-GA [Isomer 1 + 2, BD3307I, Isomer 1, Isomer 2, Glucuronic acid conjugate of AE C656948-7-hydroxy]

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



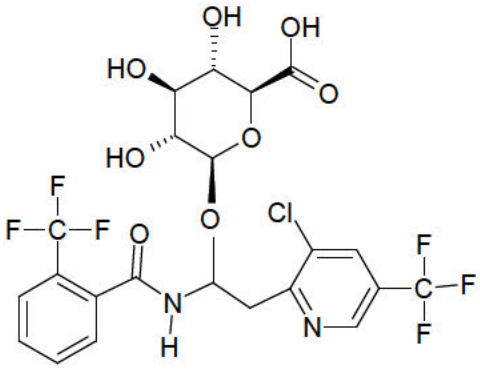
AE C656948-8-hydroxy [BD3307M1, BD3307E, BN16608A, HF3605F6]

IUPAC Name: N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]-1-hydroxyethyl}-2-(trifluoromethyl)benzamide (ISIS/Draw with ACD/Name add-in).
CAS Name: Not reported.
CAS Number: Not reported.



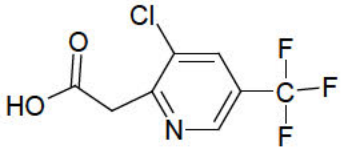
AE C656948-8-OH-GA [Isomer 2]

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



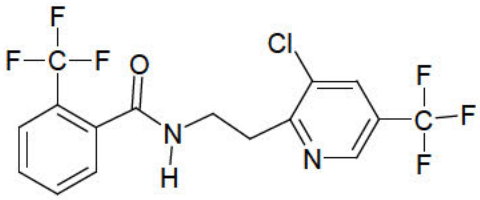
AE C656948-pyridyl acetic acid [BD3307G, BN178010]

IUPAC Name: [3-Chloro-5-(trifluoromethyl)pyridin-2-yl]acetic acid (ISIS/Draw with ACD/Name add-in).
CAS Name: Not reported.
CAS Number: Not reported.



BD3319D

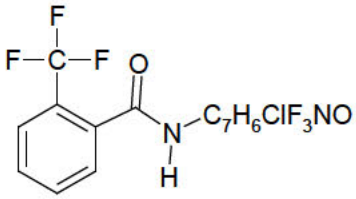
IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



+2xO, + H2O
+ Glucuronic acid - H2O

BD3319D3

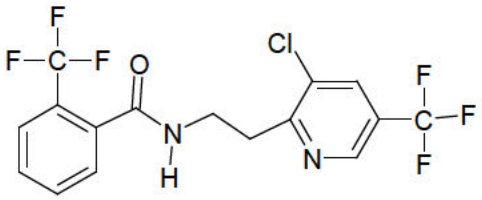
IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



+Glucuronic acid - H20

BD3319D8

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.

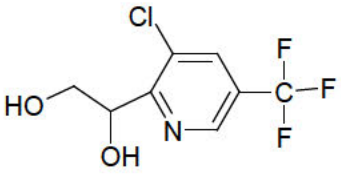


+ O, +H2O
+ Sulfuric acid - H2O

Unidentified Reference Compounds

AE C656948-ethyl-diol-GA [Isomer 3, BD3307P, BN178009]

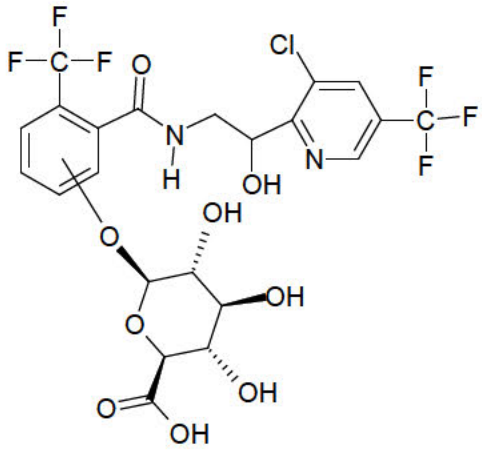
IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



+ C6H10O7
Glucuronic acid
- H2O

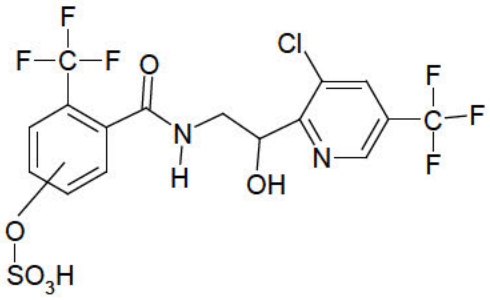
AE C656948-7-OH-phenol-GA [BD3307H, BN178015]

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



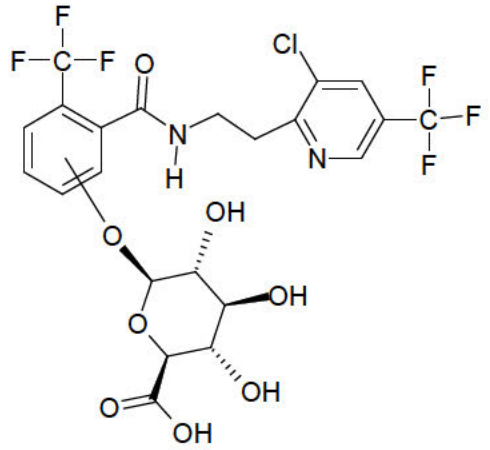
AE C656948-7-OH-phenol-SA [BD3307J, BN178022]

IUPAC Name: N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]-2-hydroxyethyl}-2-(trifluoromethyl)benzamide compound with methanol (1:1) (ISIS/Draw with ACD/Name add-in).
CAS Name: Not reported.
CAS Number: Not reported.



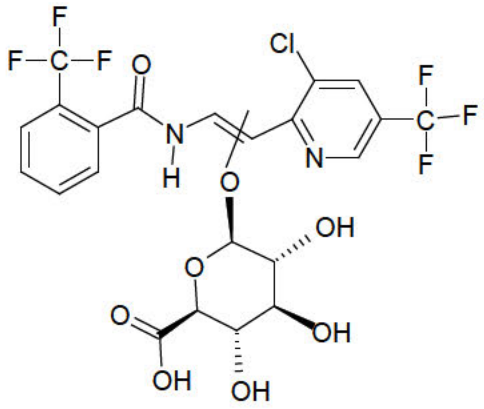
AE C656948-phenol-GA [BD3307N, Mixture with AE C656948-enol-GA, BN153007]

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



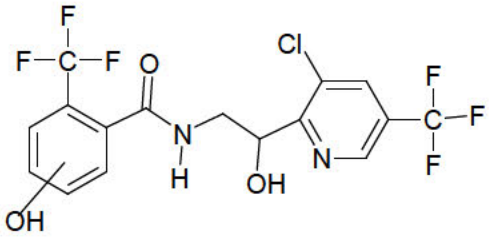
AE C656948-enol-GA, 2 isomers [BD3307K, BN178025]

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



AE C656948-7-OH-phenol [BN178026]

IUPAC Name: N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]-2-hydroxyethyl}-2-(trifluoromethyl)benzamide compound with methane (1:1) (ISIS/Draw with ACD/Name add-in).
CAS Name: Not reported.
CAS Number: Not reported.



AE C656948-8-picoline [BD3307Q1, KML3627]

IUPAC Name: 3-Chloro-2-methyl-5-(trifluoromethyl)pyridine (ISIS/Draw with ACD/Name add-in).
CAS Name: Not reported.
CAS Number: Not reported.